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A Comparison of Three Microbial Assay Procedures for Measuring Toxicity of Chemical Residues

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Abstract. Public concern about environmental and health effects from the disposal of residues from the manufacture of agricultural and industrial process chemicals into landfills led to the passage of the Comprehensive Environmental Response, Compensation and Liability Act of 1980, commonly referred to as the "Superfund" Law. One method of defining the potential toxicity of these chemical residues is the use of bioassays, a number of which use bacteria as the test organism and have been proposed to assess the impact of chemical pollutants on the environment. Efforts were directed toward the establishment of short-term methods that are inexpensive, rapid, reproducible and sensitive. A major advantage of microbial toxicity tests over chemical analysis is their direct assessement of potential biotic impact without extrapolation from chemical analysis of uncertain completeness. It was the intent of this paper to evaluate the relative sensitivity, precision, and accuracy of three published microbial bioassay procedures for ascertaining their ability to define the toxicity potential at hazardous waste sites.

Materials and Methods

Chemicals

The following chemicals were tested with all three methods: Reagent grade cupric chloride (Cu^{+2}), and pesticide grade acetone ($(CH_3)_2CO$) and methanol (CH_3OH). Some, but not all, tests were performed with reagent grade potassium cyanide (KCN), zinc chloride (Zn^{+2}), mercuric chloride (Hg^{+2}), sodium arsenite (As^{+3}), sodium arsenate (As^{+5}), arsenic trioxide (As^{+3}) and cadmium chloride (Cd^{+2}).

Toxicity Tests

The Microtox test (MTX) was performed by the procedure detailed in the Beckman[1] Microtox System Operating Manual (1982). Samples were incubated for 5, 15, and 30 min. Two replicates of each concentration were tested.

The resazurin reduction method (RR) was performed by the procedure detailed by Liu (1981). Mixed bacterial cultures were obtained from activated sludge collected at the Taylor Municipal Water Treatment Plant, Corvallis, Oregon, which does not receive wastewater from chemical or manufacturing industries. Fifty ml of 1/10 strength Difco nutrient broth, fortified with 200 mg/L each glucose and sodium acetate, were added to a 125-ml Erlenmeyer flask and inoculated with 0.1 ml of fresh activated sludge. After overnight growth on a shaker at 21°C, 0.1 ml of the culture was transferred into another flask containing fresh medium. The transfer was repeated for two weeks so that an active stabilized mixed culture was established. In addition, a pure culture of *Escherichia coli* (ATCC 10536) was assayed to permit comparison with the mixed culture (Corvallis sewage isolate = CSI) from the local sewage treatment plant. Four replicates of each concentration were tested.

The dissolved oxygen depletion (DOD) test followed the procedure outlined by Bauer (1981) with the following exceptions: (1) activated sludge was not collected for each experiment. The method of Liu (1981) was used: (2) cultures were incubated at 21 rather than 25°C; and (3) replicate concentrations were not assayed within a test, but replicate tests were performed. Each test consisted of one control and four toxicant concentrations. Dissolved oxygen was measured with Orion Research O_2 electrodes interfaced through a model 605 electrode switch that was connected to an Orion model 701a digital pH meter.

[1] Mention of trade names or products does not constitute endorsement by the Environmental Protection Agency.

Table 1. Comparison of relative toxicity (EC_{50} from eight laboratories reported as mg/L) derived from 5-minute exposures of the microtox organism *Photobacterium* sp

Toxicant	US EPA[a] 1984	Beckman 1981	Dutka et al 1981	McFeters et al 1983	Qureshi et al 1983	Curtis et al 1983	De Zwart et al 1983	Indorato et al 1983	Mean (mg/L)	Standard deviation	c.v.[b] (%)
Mercury as Hg	0.06	0.07	0.06	0.04	—	—	0.03	—	0.05	0.01	20.00
Copper as Cu	1.21	3.50	19.50	9.94	1.84	—	—	—	7.20	7.70	107.00
Sodium arsenate	3.57	—	—	—	—	—	—	—	3.57	0.00	—
Cyanide as CN	4.77	12.00	—	—	—	—	—	2.24	8.39	5.11	60.90
Zinc as Zn	11.98	26.00	14.00	152.07	55.50	—	—	—	51.91	58.63	112.95
Sodium arsenite	24.67	40.00	—	—	—	—	—	—	32.34	10.84	33.52
Arsenic trioxide	73.73	—	—	—	—	—	—	—	73.73	0.00	
Cadmium as Cd	105.59	100.00	—	255.00	154.00	—	690.00	—	260.92	247.79	94.97
Acetone	12364.00	—	—	18250.00	—	21500.00	22270.00	—	18595.00	4505.15	24.23
Methanol	158000.00	—	—	—	—	125000.00	—	—	141500.00	23334.50	16.49

[a] Results produced from this study
[b] C.V. = standard deviation/mean × 100

Statistical Analysis

Mean and standard deviations (SD) of the biological effects were calculated for replicate test concentrations. The percent effect was calculated by the following formula:

$$\% \text{ Effect} = \frac{\text{Test} - \text{Control}}{\text{Control}} \times 100$$

Linear regression analysis was performed on the data using chemical concentration (mg/l) as the x-axis and percent effect as the y-axis. An EC_{50} concentration was calculated using the equation derived in the regression analysis.

Results and Discussion

EC_{50} values of several selected toxicants are shown in Table 1. The results of 5 min exposures obtained in eight laboratories using MTX assays show there are problems of reproducibility between laboratories, *ie.*, 112.95 and 107% coefficient of variation (CV = standard deviation/mean × 100) for zinc and copper, respectively. The chemicals in Table 1 are listed in order of most to the least toxic, as determined by our studies. Regardless of the absolute EC_{50} concentration, six of the seven laboratories identified this same toxic response pattern.

Potential causes of variability in results between tests and laboratories were summarized by Dutka and Kwan (1981): (1) duration of the assay (5, 15, 30 min incubation); (2) use of different formulations of specific toxicants to yield the desired effect; (3) sample pH and solubility; (4) methods of determining toxicant concentrations (*i.e.*, measured vs calculated); (5) inaccuracies in the preparation of sample dilutions and pipetting sample aliquots; and (6) dissimilar data reduction and analysis procedures.

The variation in results caused by exposure for several chemicals is given in Table 2. All of the heavy metals and cyanide became more toxic with time. Acetone exhibited no change in toxicity from 5 to 30 min incubations. In contrast, methanol was only one-half as toxic after 15 and 30 min incubation when compared to results at 5 min. These changes may reflect lethal accumulation, kinetic effects on enzyme systems of the bacteria, or the effects of volatilization or metabolic degradation.

EC_{50} values of selected toxicants, derived from the 15 min MTX assay, are compared in Table 3. The trend in toxicity from the least to the most toxic chemicals is the same as for the 5 min exposed bacterium with the exceptions of cyanide and cadmium which became more toxic. Of particular interest is the similar precision of data obtained with 5 and 15 min MTX tests. The mean CV for all of the chemicals reported in Tables 1 and 3 for 5 and 15 min exposures are 58.9 and 57.1, respectively.

Copper was used to develop a quality control data base for all microbial tests performed in our laboratory. Results of 31 MTX tests performed by five technicians from November 1982 to August 1984 are reported in Table 4. Precision of the 5, 15, and 30 min exposures, based on their CV, are 68, 46, and 26%, respectively. Copper became more toxic with time. The EC_{50} values ranged from 1.21 to 0.23 mg copper/L for 5 and 30 min exposures, respectively. These copper response values support the data reported in Table 2, where heavy metals and cyanide were more toxic with prolonged exposure time.

Another series of tests with copper were conducted with the test developed by Liu (1981). This test is based on the reduction of resazurin by microbial dehydrogenase. The series of copper tests

Table 2. Comparison of relative toxicity for different incubation periods measured with the Beckman Microtox system

	EC_{50} (mg/L)				
	Exposure time				
Toxicant	5-Min	15-Min	30-Min	60-Min	90-Min
Mercury as Hg	0.06	0.02	0.01	—	—
Copper as Cu	1.21	0.28	0.23	0.12	—
Sodium arsenate	3.57	1.73	1.60	—	—
Cyanide as CN	4.77	1.40	0.61	—	—
Zinc as Zn	11.98	1.56	0.86	0.68	0.67
Sodium arsenite	24.67	18.39	16.60	—	—
Arsenic trioxide	73.73	43.56	33.39	31.43	—
Cadmium as Cd	102.59	25.43	13.79	3.85	2.97
Acetone	12364.00	12601.00	13213.00	—	—
Methanol	158000.00	284400.00	323900.00	—	—

Table 3. Comparison of toxicity data from different laboratories derived from 15 minute exposures in the Microtox assay

Toxicant	[a]US EPA 1984	Beckman 1981	Dutka et al 1981	Qureshi et al 1983	De Zwart et al 1983	Mean	Standard deviation	C.V.[b]
	EC_{50} (mg/L)							
Mercury as Hg	0.02	—	0.05	—	0.02	0.03	0.01	33.33
Copper as Cu	0.28	0.80	3.80	0.28	—	1.29	1.69	131.01
Sodium arsenate	1.73	—	—	—	—	1.73	0	—
Cyanide as CN	1.40	4.00	2.80	—	—	2.73	1.30	47.62
Zinc as Zn	1.56	4.00	3.50	6.08	—	3.79	1.86	49.08
Sodium arsenite	18.39	26.00	—	—	—	22.20	5.38	24.23
Arsenic trioxide	43.56	—	—	—	—	43.56	0	
Cadmium as Cd	25.43	20.00	—	41.40	140.49	56.83	56.51	99.44
Acetone	12601.00	—	—	—	28940.00	20770.50	11553.40	55.62
Methanol	158000.00	—	—	—	—	158000.00	0	16.49

[a] Results produced from this study
[b] C.V. = standard deviation/mean × 100

were performed with either a mixed culture from sewage (CSI) or a pure culture of *E. coli*. The mean EC_{50} concentration observed for copper using the CSI was 1.67 mg/L. The copper EC_{50} value for *E. coli* increased to 2.51 mg/L (Table 5). A statistical comparison of the EC_{50} values for both cultures presented in Table 5 showed differences in CV of 26.95% (CSI) and 38.65% (*E. coli*). A number of additional tests were performed to determine the cause of these variations. Five toxicity tests were performed by three different technicians and their results are shown in Table 6. The mean EC_{50} concentration for copper was 1.86 mg/L. This falls within the range of the results shown in Table 5. There was a high degree of error between the non-stressed samples as evidenced by the 129.3% CV between the five test controls. The CV for the five EC_{50} concentrations was 16.7%, indicating that regardless of the number of cells available a similar EC_{50} copper concentration effectively blocked dehydrogenase production. In the next series of tests, three technicians each added 3.5 mg copper/L to ten flasks to determine their ability to reproduce inhibitory effects from replicate concentrations. The greatest CV (Table 7) was 4.45%, and the mean CV for the three technicians' EC_{50} values was 3.34%. These data support our previous observation that EC_{50} or greater concentrations of copper mask the variation between replicates.

Escherichia coli had a bi-modal response to the copper (Figure 1). There was a linear decline in dehydrogenase production with increase in copper concentration up to 2 mg/L ($r^2 = 0.95$), but from 2 to 12 mg/L the biological effect of copper plateaued at 60.6% inhibition. Copper toxicity again increased from 12 to 32 mg/L until 98% inhibition was

Table 4. Toxicity of Copper (as Cu^{++}) in the Microtox Assay after exposures from 5 to 60 minutes

Test number	Date	Technician[a] number	EC_{50} (mg/L) Exposure time 5-Min	15-Min	30-Min	60-Min
1	11/18/82	5	3.00	0.19	0.29	—
2	B	5	2.93	0.24	0.29	—
3	C	5	—	0.16	0.24	—
4	D	5	—	0.20	0.23	—
5	E	5	—	0.21	0.24	—
6	F	5	—	0.22	0.21	—
7	G	5	—	0.37	0.22	—
8	H	5	—	0.43	0.27	—
9	I	5	—	—	0.26	—
10	J	5	—	—	0.29	—
11	K	5	—	—	0.27	—
12	L	5	—	—	0.25	—
13	M	5	—	—	0.32	—
14	12/12/82	5	1.21	0.55	—	—
15	12/17/82	5	0.92	0.33	—	—
16	12/20/82	5	1.27	0.29	0.20	0.15
17	12/27/82	5	0.69	0.24	0.19	—
18	B	5	1.19	0.34	—	—
19	06/20/83	5	1.21	0.60	—	—
20	B	5	0.75	0.35	—	—
21	06/23/83	5	1.59	0.47	0.28	—
22	B	5	1.58	0.35	—	—
23	01/18/84	4	2.21	0.30	0.23	—
24	B	4	1.78	0.36	0.23	—
25	07/18/84	3	0.22	0.13	—	—
26	07/19/84	1	0.66	0.13	0.08	—
27	07/20/84	2	0.38	0.13	0.12	—
28	07/23/84	1	0.41	0.13	0.12	—
29	08/24/84	2	0.59	0.19	—	—
30	B	2	0.39	0.07	—	—
31	08/28/84	2	—	0.31	0.17	0.10
Minimum			0.22	0.07	0.08	0.10
Maximum			3.00	0.60	0.33	0.15
Mean			1.21	0.28	0.23	0.12
S.D.			0.82	0.13	0.06	0.03
C.V. (%)[b]			67.77	46.43	26.09	25.00

[a] Each of the five technicians was assigned a number to allow for comparison of their individual test results
[b] C.V. = standard deviation/mean

achieved at 32 mg/L. An important observation from this information is that when the copper concentration is below 2 mg/L there is a marked increase in the CV with decreasing copper concentration.

Six tests were performed by one technician, using CSI, (Table 8) that resulted in an EC_{50} of 2.24 mg copper/L and a goodness of fit (r^2) of 0.85. These tests demonstrated an increase in the CV with decrease in copper concentration. Table 9 illustrates similar results produced by another technician using *E. coli*.

Six additional metal salts and organic compounds were tested for toxicity by the RR inhibition method. The results (Table 10) follow the same order (from least to most toxic) as that shown in Table 1 for the MTX tests. The RR sensitivity was similar to MTX for methanol tested with *E. coli*, but was 23 times less sensitive than the MTX assays in predicting toxicity of cadmium. The RR EC_{50} concentrations reported in Table 11 compare favorably with the results published by Liu (1981) and Gillett *et. al.* (1983). They are certainly no worse than the literature comparisons for MTX illustrated in Table 2. The results of the RR copper test series demonstrate that there is a remarkable degree of

Table 5. The effect of copper on pure and mixed bacterial cultures using Liu's resazurin reduction method at 21° C

Mixed culture			*E. coli* (ATCC 10536)	
	mg/L			
EC_{50}		r^2	EC_{50}	r^2
	2.606	0.77	4.355	0.91
	2.133	0.65	3.212	0.88
	1.364	—	2.265	0.95
	1.574	—	2.096	0.86
	1.511	0.98	1.797	0.93
	1.401	0.83	2.325	0.77
	1.236	0.86	1.512	0.96
	1.194	0.72	—	—
	1.311	0.94	—	—
	1.386	0.74	—	—
	2.278	0.82	—	—
	1.999	0.81	—	—
	1.733	0.68	—	—
Summary:				
Mean	1.67	0.80	2.51	0.89
Standard deviation	0.45		0.97	
C.V. (%)[a]	26.95		38.65	

[a] C.V. = standard deviation/mean × 100

Table 6. Comparison of the effect of technicians on the EC_{50} of copper measured by the resazurin reduction assay

Technician	mg Copper/L		
	EC_{50}	r^2	D.F.
3	2.265	0.95	2
2	2.096	0.86	3
3	1.638	0.98	3
1	1.797	0.93	4
1	1.512	0.96	3
Summary:			
Mean	1.86	0.94	
Standard deviation	0.31		
C.V. (%)[a]	16.67		

[a] C.V. = standard deviation/mean × 100

repeatability of the EC_{50} concentrations in spite of the great variability of results at low copper concentrations. Both the *E. coli* and CSI appear unable to produce quantities of dehydrogenase consistent with the concentration of copper to which they were exposed. However, because there was a plateau in the responses of the bacteria to copper concentrations from 2 to 12 mg/L, the EC_{50} values would always fall near 2 mg/L, regardless of the slope of the line calculated by linear regression analysis (Figure 1). Copper toxicity results measured by the DOD method of Bauer *et al.* (1981), are illustrated in Table 12. Five tests were conducted to define the reproducibility of the test method. Unlike Bauer (1981), who used bacteria

Table 7. Pipetting reproducibility among three technicians using copper

Mg Cu/L	% Effect	Standard deviation	C.V.[a] (%)	D.F. (N-2)	Technician
3.5	−66.15	1.75	2.65	7	1
3.5	−70.34	3.13	4.45	8	2
3.5	−67.77	2.71	3.99	8	3
Summary:					
Mean % Effect	−67.75				
S.D.	2.26				
C.V.	3.34%				

[a] C.V. = standard deviation/mean × 100

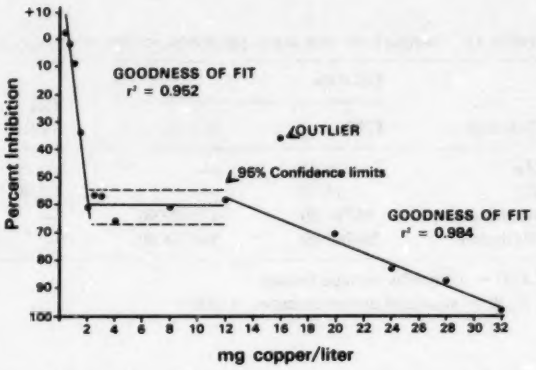

Fig. 1. The effects of copper on *E. coli* measured by the Resazurin Reduction method

Table 8. Variation in copper EC_{50} results in tests performed by technician Number 5 with mixed bacterial cultures

Mg Cu/L	% Effect	Standard deviation	C.V.[a] (%)	D.F. (N-2)
0.25	−8.1	3.61	44.57	1
0.50	−25.2	11.65	46.23	3
1.00	−39.1	14.09	35.99	13
2.50	−69.2	7.45	10.77	13
5.00	−80.8	5.89	7.29	10
10.00	−81.2	6.76	8.33	8
Summary:				
EC_{50}	2.24 mg Cu/L			
r^2	0.85			
D.F.	3.00			

[a] C.V. = standard deviation/mean × 100

Table 9. Two tests performed by technician Number 3 using pure cultures

Mg Cu/L	% Effect	Standard deviation	C.V.[a] (%)	D.F. (N-2)
0.38	0.05	0.92	1838.48	0
0.75	−2.05	0.64	31.04	0
1.00	−8.20	14.14	172.47	0
1.50	−35.65	28.78	80.73	0
2.00	−61.80	2.02	3.27	0

Summary:

EC_{50}	1.83 mg Cu/L
r^2	0.94
D.F.	3.00

[a] C.V. = standard deviation/mean × 100

Table 10. Comparison of toxicity for several chemicals measured by the resazurin reduction assay (RR)

Toxicant	CSI[b]	Ratio[a] RR/CSI	EC_{50} (mg/L) E. coli	Ratio RR/CSI
Mercury as Hg	0.91	15.00	—	—
Copper as Cu	1.67	1.40	2.51	2.1
Cyanide as CN	14.77	3.10	--	—
Zinc as Zn	264.88	22.00	—	—
Cadmium as Cd	2400.00	23.00	—	—
Acetone	123640.	7.80	129489.	10.5
Methanol	264765.	1.70	144534.	0.9

[a] The ratio is the EC_{50} value of the resazurin reduction divided by EC_{50} value from the microtox assay results
[b] CSI = Corvallis sewage isolate

Table 11. Comparison with other published results of toxicity (mg/L) found by a 90-minute resazurin reduction assay

Toxicant	US EPA CSI[a]	US EPA *E. coli*	Liu 1981	Gillett et al 1983	Mean	S.D.	C.V.[b] (%)
Hg^{++}	0.91	—	0.75	—	0.83	0.11	13.3
CN^-	14.77	—	28.00	—	21.39	9.36	43.8
Acetone	96394.00	129489.00	—	98000.00	107961.00	18661.10	17.3
Methanol	264765.00	144534.00	—	96000.00	168433.00	86883.70	51.6

[a] CSI = Corvallis sewage isolate
[b] C.V. = standard deviation/mean × 100

from isolated fresh sewage, we chose to use pure cultures of *E. coli* for this portion of our research.

The tests spiked with 3.5 mg copper/L had CV between replicate flasks from 4 to 63%, with a four-test average mean of approximately 60%. This wide range in accuracy is attributed to the inconsistency of the response of the bacteria to the toxin at low concentrations rather than technician error. The 19.36% inhibition caused by 3.5 mg copper/L measured by DOD is similar to that obtained with 1.5 mg copper/L measured by RR (Table 9). Although the sensitivity of the DOD method to measuring copper toxicity is less than RR the repeatability of effects at low and mid-range (5 mg/L) copper concentrations is much better. In the 11 tests shown in Table 12, each technician had a test whose mean effect was substantially different than the others at that same copper concentration. However, at each concentration, the test with the unusual results was performed by a different technician. For example, the second test by technician T-3 yielded 36.65% inhibition at a concentration of 3.5 mg copper/L. The mean EC_{50} value of the preceding three tests was 13.6% inhibition or only 37% as inhibited as the results from the last test by T-3. A similar pattern occurs for technicians T-2 at 5.0 mg/L and T-1 at 7.0 mg/L. Furthermore, comparison of the two tests spiked with 3.5 mg/L performed by T-3 produced a mean effect of 25.6% inhibition with a standard deviation of 15.63. The 61.05% CV calculated from these results matches with the 57.6 and 62.9% CV from T-1 and T-2, respectively.

Dissolved oxygen depletion results of extended exposures of *E. coli* to copper (Table 13) displayed the same pattern of erratic and unpredictable recovery rates that are seen in other tests of sublethal concentrations of toxicants. The 3.5 mg/L concentration of copper that produced 19.36% inhibition with a brief exposure produced 511.7% more DOD (stimulation) than the controls after extended exposure. On the other hand, the 5.0 mg/L concentration had exceeded the ability of the system to detoxify the copper and the extended inhibition was greater (93.1%) than the brief exposure effects (19.36%). Comparison of the brief and extended exposure DOD results are reported in Table 14. In spite of the variations of results within replicate flasks and between tests, the EC_{50} concentrations for both brief and extended exposures are remarkably close. The goodness of fit (r^2) values for the

Table 12. Comparison of the reproducibility of results within and between tests and technicians (T) using the dissolved oxygen depletion method and a brief exposure (30 min) of *E. coli*

Copper	Replicate number	T-1	T-2	T-3	T-3	Mean of all tests
3.5 mg/L	1	−7.30	−25.00	−15.20	−29.10	—
	2	−17.10	−10.20	−14.50	−33.30	—
	3	−6.30	−11.40	−14.70	−38.50	—
	4	−21.80	−5.90	−13.80	−45.70	—
	Mean	−13.13	−13.13	−14.55	−36.65	−19.36
	S.D.	7.56	8.26	0.58	7.15	11.54
	C.V. (%)	−57.58	62.91	3.99	19.52	59.61
5.0 mg/L	1	−39.90	−22.30	−52.80	—	—
	2	−43.90	−16.00	−55.60	—	—
	3	−58.80	−12.80	−51.40	—	—
	4	−43.20	−17.00	−45.80	—	—
	Mean	−46.45	−17.03	−51.4	—	−38.29
	S.D.	8.42	3.95	4.12	—	18.38
	C.V. (%)	18.12	23.18	8.02	—	57.54
7.0 mg/L	1	−82.90	−61.40	−62.80	−65.40	—
	2	−83.80	−58.90	−59.10	−65.10	—
	3	−84.20	−64.60	−58.00	−53.50	—
	4	−87.30	−61.80	−53.80	−62.60	—
	Mean	−84.53	−61.68	−58.43	−61.65	−66.57
	Standard deviation	1.94	2.33	3.70	5.58	12.07
	C.V. (%)[a]	2.30	3.79	6.34	9.05	18.13

[a] C.V. = standard deviation/mean × 100

Table 13. Comparison of the effects of copper in extended dissolved oxygen depletion tests using *E. coli*

	Extended exposures			
Concentration (mg/L)	Mean % effect	Standard deviation	C.V.[a] (%)	D.F. (N-2)
3.50	+511.70	504.9	98.7	14
5.00	−93.10	6.8	7.3	19
7.00	−81.00	40.3	49.7	10

[a] C.V. = standard deviation/mean × 100

brief exposures are very good at 0.70 and above. However, the lower r^2 values in the extended tests reflect the erratic behavior of the cultures attempting to overcome the toxicant effects.

Bauer *et al.* (1981) reported their data using activity quotients (AQ). Since the activity quotient has little value in our method of reporting data we converted Bauer's data (1981, Figure 2) to EC_{50} values. The conversion produced brief and extended exposure EC_{50} values of 4.62 and 2.33 mg copper/L, respectively. Their brief exposure results agree very well with ours, but their extended exposure results are less than half the concentration that we reported. Another association that can be made to Bauer's (1981) work is to compare the activity quotient for 5 mg copper/L (Bauer *et al.* 1981, Table 1) to our results for the same concentration. Bauer *et al.* (1981) reported an AQ of .43. The reciprocal of the AQ times 100 converts that AQ to percent effect. The resultant transformation to 57% inhibition compares well with the results of technicians T-1 and T-3, which reported 46.45 and 51.4% inhibition, respectively.

A number of toxicants were tested with the DOD test using the bacterial cultures isolated from the Corvallis waste treatment plant (Table 15). As was the case with MTX and RR, the acetone and methanol tests demonstrated the lowest toxicity. The EC_{50} value of 6.63 mg/L for copper was also reasonable compared to the results determined from the tests using *E. coii*. However, the pattern of metal toxicity was different from our other observations, with copper the least and zinc the most toxic.

Table 14. EC_{50} concentrations (mg Cu/L) for brief and extended dissolved oxygen depletion tests and comparison of results of three tests performed by three technicians

Technician	EC_{50}	r^2	D.F.	Summary Data	
	Brief Exposure				
1	5.26	0.96	10	Mean	5.78
2	6.48	0.87	14	Standard deviation	0.63
3	5.59	0.70	14	C.V.[a]	10.90%
	Extended Exposure				
1	4.85	0.60	6	Mean	4.87
2	4.96	0.82	6	Standard deviation	0.09
3	4.79	0.57	10	C.V.	1.80%

[a] C.V. = standard deviation/mean × 100

Table 15. Comparison of the relative toxicity of chemicals (EC_{50}) to CSI given brief and extended exposures and measured by the dissolved oxygen depletion method

Chemical (mg/L)	Brief	Extended
Zinc as Zn	0.22	>10.00
Mercury as Hg	0.25	0.80
Cyanide as CN	3.48	23.32
Copper as Cu	6.63	>8.00
Acetone	68112.00	10202.00
Methanol	16669.00	33486.00

Table 16. The effects of copper (EC_{50} = mg/L) on bacteria and a comparison of the sensitivities of DOD and RR to Microtox

Method & organism	Exposure time					Microtox Ratios[a]	
	5-Min	15-Min	30-Min	90-Min	22-Hr	Short	Long
Microtox	1.210	0.281	0.227	—	—	1	1
DO Depletion							
E. coli	—	—	5.780	—	4.87	5	22
CSI[b]	—	—	6.630	—	>8.00	6	—
Resaz. Reduction							
E. coli	—	—	—	1.67	—	1	7
CSI	—	—	—	2.51	—	2	11

[a] Short-Term ratios are the 30-Min DO and 90-Min RR EC_{50} divided by the 5-Min Microtox EC_{50}. Long-Term Ratios are the 22-HR DO and the 90-Min RR EC_{50} divided by the EC_{50} of the 30-Min Microtox Test.
[b] CSI = Corvalis sewage isolate

Table 17. The effects of acetone (EC_{50} = mg/L) on bacteria and a comparison of their sensitivities to Microtox

Method & organism	Exposure Time					Microtox Ratios[a]	
	5-Min	15-Min	30-Min	90-Min	22-Hr	Short	Long
Microtox	12364	12601	13213	—	—	1	1
DO Depletion							
E. coli	—	—	68112	—	10202	6	1
Resaz. Reduction							
E. coli	—	—	—	96394	—	8	7
CSI[b]	—	—	—	129489	—	11	10

[a] Short-Term Ratios are the 30-Min DO and 90-Min RR divided by the 5-Min Microtox Test Results. Long-Term Ratios are the 22-Hr DO and the 90-Min RR divided by the results of the 30-Min Microtox Test Result
[b] CSI = Corvallis sewage isolate

Our concern for finding a way to sterilize the dissolved oxygen probes caused us to contact the technical services staff at Orion Industries. We learned that there is no accepted means for sterilizing the probes. Of greater concern was the information that we obtained about the chemical poisoning of the probes. Poisoning can be caused by: (1) any wetting agent or detergent; (2) any surfactant; (3) grease and oil that coats the membrane; and (4) methanol and acetone. Acetone, in fact, is extremely toxic to the Orion DO probes. With this information in hand, the results of tests using this equipment are suspect. Since probes can be poisoned by many substances the DOD assay should be ruled out as an acceptable approach for measuring the effects of environmental samples suspected of containing complex waste mixtures.

The EC_{50} concentrations of copper for all of the

Table 18. The effects of methanol (EC_{50} = mg/L) on bacteria and a comparison of their sensitivities to Microtox

Method & organism	Exposure Time					Microtox Ratios[a]	
	5-Min	15-Min	30-Min	90-Min	22-Hr	Short	Long
Microtox	158000	284400	323900	—	—	1.0	1.0
DO Depletion							
E. coli	—	—	166690	—	33486	1.1	0.1
Resaz. Reduction							
E. coli	—	—	—	144534	—	0.9	0.5
CSI	—	—	—	264765	—	1.7	0.8

[a] Short-Term Ratios are the 30-Min DO and 90-Min RR EC_{50} Divided by the 5-Min Microtox EC_{50}. Long-Term Ratios are the 22-HR DO and the 90-Min RR EC_{50} Divided by the EC_{50} of the 30-Min Microtox Test.
[b] CSI = Corvallis sewage isolate

assays performed are shown in Table 16. The sensitivity of MTX assays to copper was compared to the sensitivities of the other test methods and organisms. Only *E. coli* measured by the RR assay was similar in sensitivity to the 5 min MTX assay. However, the 30 min exposure MTX assay was 5 times more sensitive to copper than the RR assay. Microtox assays are 2 to 22 times more sensitive to copper than the other microbial assays investigated. The DOD test was the least sensitive to copper either for brief or extended exposures.

The results measured by the MTX assay to acetone were 7 to 11 times more sensitive than were those reported by RR (Table 17). DOD was more sensitive to acetone than RR, but the possibility of probe poisoning cast suspicion on the scientific credibility of the results.

Comparison of all test results for methanol (Table 18) showed that, although the question of DOD probe poisoning remains, the average effective concentrations for all three assays were quite similar. The only exception was the DOD extended exposure results which were 10 times more sensitive than those derived from 30 minute MTX assays.

The data presented in summary Tables 16, 17, and 18 for copper, acetone and methanol, respectively, show MTX to be more reliable and sensitive to these chemicals than RR and DOD microbial tests. The advantages of standardization and our ability to verify test results with those of other researchers has led us to reject the RR and DOD tests as candidates for assessment of chemical contamination from hazardous waste sites.

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